

They note that this inflammatory reaction at times failed to occur, and in this our results agree.

The conclusion at which the observers named arrive is that the subcutaneous tissues of the guinea-pig are peculiarly sensitive to adrenal grafts, which produce in them œdema and hæmorrhagic solution.

It is difficult, however, to eliminate here the possibility that the results are due to auto-bacterial infection.

The organs of normal guinea-pigs abound in various forms of bacteria, the growth of which is inhibited under natural conditions. When necrosis is set up by the adrenal graft or by the injection of an extract, the dead tissues furnish a nidus in which the latent pathogenic micro-organisms may grow.

Further Results of the Experimental Treatment of Trypanosomiasis: being a Progress Report to a Committee of the Royal Society.

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The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'*

The experiments have been carried out with the same strains of Nagana and Surra as were used before.

A.—Condition of the Animals living at the Date of the Completion of the Tables in the last Paper.

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury.

(Average duration of untreated disease, 5·5 days.)

No.	4	died on the	307th	day after inoculation.
„	7	„	365th	„
„	10	„	249th	„
„	15	„	188th	„
„	21	„	63rd	„

Of these, No. 15, which was apparently cured, was used on the 147th day after inoculation for re-inoculation, with the view of ascertaining if any immunity had been conferred. This was found not to be the case.†

* B, vol. 79, 1907, pp. 505—516, and B, vol. 80, 1908, pp. 1—12.

† *Vide* 'Roy. Soc. Proc.,' B, vol. 80, p. 10.

None of the above died with any of the signs of Nagana. Of the 21 rats tabulated,* only one died from trypanosomiasis, and this one was probably atoxyl-proof.

Table III.—Surra Rats treated with Atoxyl and Mercury Sozoiodol.

No. 5 died on the 286th day after inoculation from broncho-pneumonia.

Table VI.—Surra Rats treated with Atoxyl and Iodopin.

No. 9 died on the 221st day after inoculation from worms.

Rats treated with Atoxyl and Liq. Hydrarg. Perchlor.

The rat still living at the date of the last paper died on the 187th day after inoculation from broncho-pneumonia.

Rats treated with Sodium Antimonyl Tartrate. (Table on pp. 8—9 of the last Paper.)† Results to August 20.

No. 7 is living and well 354 days after inoculation.

„	8	died on the 194th day	„
„	9	323rd „	„
„	10	145th „	„
„	14	48th „	„
„	15	67th „	„
„	16	227th „	„
„	17	227th „	„
„	19	161st „	„
„	20	49th „	„
„	21	210th „	„
„	23	104th „	„
„	25	134th „	„
„	26	134th „	„
„	27	33rd „	„
„	28	34th „	„
„	29	57th „	„
„	30	28th „	„
„	31	147th „	„
„	32	is living and well 346 days after inoculation.	
„	33	died on the 228th day	„
„	34	188th „	„
„	35	is living and well 346 days after inoculation.	
„	36	died on the 228th day after inoculation.	„
„	38	173rd „	„

* 'Roy. Soc. Proc.,' B, vol. 79, p. 510.

† 'Roy. Soc. Proc.,' B, vol. 80.

It will be noticed that nine of the above rats lived for over 200 days, and nine others considerably over 100 days. Of those which have died only four have had recurrences (two had one, and two had three recurrences); none of them died with any symptoms of trypanosomiasis, and in none were trypanosomes found after death. An emulsion of the liver and of the bone-marrow of Nos. 9, 16, 17, 21, and 25 was injected into other rats with negative results in every case.

Rats treated with equal parts of 1 per cent. Solutions of Sodium Antimonyl Tartrate and Sodium Arsenyl Tartrate.

The five rats living at the date of the last paper have died :—

No. 1 died on the 199th day after inoculation.

„ 2	„	26th	„
„ 3	„	43rd	„
„ 4	„	59th	„
„ 5	„	40th	„

As was anticipated from the earlier experiments, this treatment has no advantages over that with antimony alone.

B.—Further Experiments.

Potassium Antimonate.

This was tried in doses up to 7 minims of a 1 per cent. solution. In this dose it was poisonous to rats, and it did not kill the trypanosomes.

Atoxyl and Sodium Antimonyl Tartrate.

This was given in doses of 5—7 minims of a 5 per cent. solution of atoxyl and of a 1 per cent. solution of sodium antimonyl tartrate. Three Nagana rats lived respectively 23, 43, and 41 days; they all had recurrences (the last had five) and died with living trypanosomes in the blood.

Pushing the drug does not have any good effect (see next table); the trypanosomes are not driven out more quickly, or more effectually; recurrences are more common, and inflammatory intestinal lesions were present in nearly every case. Two rats died with living trypanosomes in the blood: these two had become antimony-proof, as the later doses did not remove the trypanosomes from the blood, nor make any difference in their number. This strain did not maintain this quality for long, as after the second sub-inoculation from these rats the trypanosomes seemed to have become normal in their behaviour towards antimony. The strain was lost at this point.

Nagana Rats treated with Large Doses of Sodium Antimonyl Tartrate.

No.	Weight in grammes.	Total quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
1	200	5·75	1	Died on 30th day with intestinal lesions.
2	125	5·0	0	Died on 34th day with very marked intestinal lesions.
3	100	4·75	0	Died on 24th day: intestines necrotic.
4	100	4·0	0	Died on 108th day from pneumonia.
5	110	4·9	1	Died on 43rd day with inflamed intestines.
6	110	6·5	2	Died on 38th day with necrotic intestines.
7	175	8·5	2	Died on 40th day: trypanosomes in blood at death; paralysed.
8	110	9·0	2	Died on 45th day in similar condition to No. 7.
9	150	5·0	4	Died on 73rd day from gangrene of tail.
10	175	5·25	1	Died on 64th day from pneumonia.

Rats treated with Sodium Antimonyl Tartrate after Inoculation with Atoxyl-proof Trypanosomes.

The rats in the following table were all treated firstly with atoxyl to make sure that they were atoxyl-proof, and the treatment with antimony was then begun, when the trypanosomes were in large numbers in the blood.

	No.	Weight in grammes.	Total quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
Nagana....	1	100	3·0	1	Died on 29th day with trypanosomes in blood.
	2	100	1·0	0	Died on 11th day from nephritis.
	3	110	3·0	1	Died on 54th day.
	4	110	3·0	1	Died on 20th day with trypanosomes in blood.
	5	175	3·0	0	Died on 117th day from broncho-pneumonia.
Surra.....	6	100	3·0	0	Died on 27th day from septicaemia.
	7	145	5·75	1	Died on 41st day from pneumonia.
	8	140	4·0	1	Died on 38th day with trypanosomes in blood.

It will be seen that the atoxyl-proof strains of trypanosomes are less influenced by antimony than are the ordinary variety, as three of the above rats had living trypanosomes in the blood at death, and seven of them died at a very early date.

*Rats treated with Antimony (Metal) and Sodium Antimonyl Tartrate
suspended in a Fatty Medium.*

Sodium antimonyl tartrate, when injected in watery solution into man produces very severe pain and inflammation in the neighbourhood of the injection, with more or less local necrosis. In order to make the use of antimony practicable in the form of injection, a series of experiments was undertaken, using various other media than water for solution or suspension of the antimony salt. Lanolin, olive oil, and sesamum oil were tried, but the results were not good. Finally, the medium Colonel Lambkin devised,* consisting of palmitin and antiseptics, which is used very largely for the intramuscular injection of calomel and mercury in syphilis, was tried, with the results which are set forth in the tables below.

One great advantage of these preparations is that they can be used upon man with far less difficulties and after-consequences than the watery solutions, which seem to be impracticable; this is of importance should antimony be found of use in human trypanosomiasis.

Major Ward, R.A.M.C., has used both the forms mentioned above on men for other purposes, and he has very kindly placed his notes at our disposal. In one of his cases four doses of $\frac{1}{2}$ grain of sodium antimonyl tartrate, suspended in Colonel Lambkin's medium, were given intramuscularly into the buttock, the intervals between the doses being 3, 4, and 3 days: the doses were then increased to 1 grain, and seven doses of this strength were given, the intervals being 4, 3, 2, 4, 2, and 13 days. In all, this patient had 9 grains of the salt. Major Ward says that "the injections caused a certain amount of tenderness and discomfort at the seat of injection," but that is very different to the effects noticed after the injection of the watery solution.

Major Ward also treated two patients with antimony itself in a state of extremely fine division suspended in Colonel Lambkin's medium; they were each given one dose of 1 grain, and 11 days afterwards $\frac{1}{2}$ grain. This form caused both pain and discomfort, and also a general increase in the size of the buttock, into which the injection was made, but this subsided without suppuration. This form would appear, however, to be much the more powerful of the two, as the effects obtained from the $1\frac{1}{2}$ grains of the metal were as good and as lasting as those observed in the case in which 9 grains of sodium antimonyl tartrate were given.

The following tables show the results obtained in rats with sodium antimonyl tartrate and antimony prepared as mentioned above:—

* 'Journ. Roy. Army Med. Corps,' 1906.

Sodium Antimonyl Tartrate, 5 per cent., in Colonel Lambkin's Medium.

	No.	Weight, in grammes.	No. of doses given.	Total quantity given, in minims.	Recur- rences.	Results to August 20.
Nagana.	1	100	4	18	2	Died on 53rd day : bad itch.
	2	100	2	6	1	Died on 16th day with intestinal lesions.
	3	115	1	2	0	Died on 113th day of pneumonia.
	4	150	1	2	0	Died on 19th day of pneumonia.
	5	110	5	17	1	Died on 27th day : abscess.
	6	125	3	13	0	Died on 26th day of pneumonia.
	7	150	3	13	0	Died on 89th day : itch.
	8	175	1	3	1	Died on 24th day : abscess.
Surra.	9	200	3	17	1	Died on 40th day of injury.
	10	225	3	13	2	Died on 28th day with intestinal lesions.
	11	115	2	6	1	Died on 22nd day of pneumonia.
	12	220	11	33	7	Died on 66th day with acute intestinal lesions.
	13	125	1	3	0	<i>Living and well 154 days after inoculation.</i>

None of the above have died from trypanosomiasis. In rats the intra-muscular injection of even a few minims is difficult, and if any of the material be left under the skin a slough forms, which takes a long time to heal. It will be noticed that No. 3 had only one dose: the rat lived 113 days and died of pneumonia; inoculations made from its organs were negative. No. 13, which is still living (154 days), has also had only one dose.

From the following table it will be seen that the administration of the metal itself has a considerable effect on the trypanosomes: it has a distinctly better effect on Surra than upon Nagana, four Surra rats out of 16 being still alive, and four others having lived for a long time. In none of the Surra rats were trypanosomes found at death, whereas in three of the Nagana rats they were present. The metal is much more irritating than the tartrate, but the effect is in most cases more prolonged; this is probably due to the fact that the absorption of the metal is much slower. Further, the smaller doses would appear to be the most efficient.

Antimony, in a state of very fine division, 5 per cent., in Colonel Lambkin's Medium.

	No.	Weight, in grammes.	No. of doses given.	Total quantity given, in minims.	Recur- rences.	Results to August 20.
Nagana.	1	100	3	12	3	Died on 61st day : abscess.
	2	200	3	11	2	Died on 29th day with trypanosomes in blood.
	3	100	3	9	1	Died on 34th day with intestinal lesions.
	4	110	3	12	1	Died on 15th day with trypanosomes in blood.
	5	100	4	14	1	Died on 27th day with intestinal lesions.
	6	150	4	15	2	Died on 26th day with intestinal lesions.
	7	150	6	23	3	Died on 33rd day with trypanosomes in blood.
	8	300	3	13	4	Died on 51st day from broncho-pneumonia.
	9	300	2	8	2	Died on 36th day from broncho-pneumonia.
	10	200	2	8	0	<i>Living and well 204 days after inoculation.</i>
	11	300	2	8	1	Died on 29th day : abscess.
	12	250	2	8	1	Died on 39th day from broncho-pneumonia.
	13	250	2	8	7	Died on 92nd day from pneumonia.
	14	300	1	5	0	Died on 32nd day : fatty liver.
	15	300	1	5	0	Died on 30th day.
	16	150	1	3	0	Died on 74th day from pneumonia.
Surra.	17	110	1	5	0	<i>Living and well 230 days after inoculation.</i>
	18	125	4	17	4	Died on 146th day : one recurrence took place after an interval of 12 weeks.
	19	250	4	35	3	Died on 48th day from broncho-pneumonia.
	20	225	1	4	0	Died on 39th day from broncho-pneumonia.
	21	100	1	4	0	Died on 44th day : itch.
	22	100	3	10	1	Died on 44th day : itch.
	23	150	2	9	1	Died on 216th day of pneumonia.
	24	100	3	12	3	Died on 94th day : abscess. One recurrence took place after an interval of 46 days.
	25	100	2	9	0	<i>Living and well 253 days after inoculation.</i>
	26	150	2	9	0	Died on 29th day from pneumonia.
	27	125	1	3	0	<i>Living and well 226 days after inoculation.</i>
	28	100	1	3	0	Died on 91st day : itch.
	29	100	1	3	0	<i>Living and well 226 days after inoculation.</i>
	30	125	1	4	0	Died on 27th day from pneumonia.
	31	100	1	4	0	Died on 27th day from pneumonia.
	32	150	1	4	0	Died on 41st day : itch.

Experiments in which Antimony and Sodium Antimonyl Tartrate were given before Inoculation, in order to Test their Effects upon the Development of the Disease.

In these experiments the substances were given suspended in Colonel Lambkin's medium, and it will be noticed that the metal is far more effective in delaying the appearance of the trypanosomes in the blood than is the salt: this is probably due to the slower elimination of the metal. This method, if the doses were repeated, might be of some practical value in getting animals safely across dangerous tracts of country.

Four Rats were given 5 minims of 5 per cent. Antimony (metal) Cream on December 19. In Nagana the trypanosomes usually appear in the blood on the second or third day after inoculation.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20.....	December 31, the 11th day.
2	„ 21.....	January 2, the 10th day.
3	„ 23.....	December 31, the 8th day.
4	„ 24.....	„ 30, the 6th day.

Four Rats were given 3 minims of 5 per cent. Sodium Antimonyl Tartrate Cream on December 19.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20.....	December 25, the 5th day.
2	„ 21.....	„ 24, the 3rd day.
3	„ 23.....	„ 26, the 3rd day.
4	„ 24.....	„ 27, the 3rd day.

Rats treated with Lithium Antimonyl Tartrate.

There are differences in the effects produced by the potassium, sodium, and lithium antimonyl tartrates, if given under similar conditions and dosage. The commercial potassium salt is very impure. The pure sodium and lithium salts which we have used have been prepared for us by Dr. R. H. Aders Plimmer, of University College. The sodium salt contains roughly about 2 per cent. more antimony than the potassium salt, and the lithium salt contains about 2 per cent. more antimony than the sodium; but the doses of the lithium salt have to be much smaller than

the corresponding doses of the sodium salt. For instance, 0·5 c.c. of a 1 per cent. solution of the lithium salt is fatal to a rat of 125 grammes, and 0·39 c.c. is fatal to a rat of 80 grammes. When the watery solution is injected intramuscularly, it has not caused necrosis of the tissues in rats, but subcutaneously it has occasionally done so. We have found that the best strength of solution for rats is 0·25 per cent., and of this 0·5 c.c. has been given for a dose.

The following table shows the effects of this dosage.

	No.	Total quantity in c.c. given of 0·25 per cent. solution.	Recurrences.	Results to August 20.
Nagana...	1	7	2	Died of disease on 47th day.
	2	7	1	Killed on 47th day, owing to abscess. Emulsion of organs injected into another rat gave negative result.
	3	4·5	1	Allowed to die on 34th day, the date of recurrence.
	4	4·5	0	<i>Alive and well 134 days after inoculation.</i>
	5	4	0	<i>Alive and well 125 days after inoculation.</i>
Surra.....	6	4	0	<i>Alive and well 134 days after inoculation.</i>
	7	4	0	Died on 42nd day of broncho-pneumonia : no evidence of trypanosomes.
	8	4	0	<i>Alive and well 134 days after inoculation.</i>
	9	5	2	Died of disease on 47th day.
	10	4·5	0	<i>Alive and well 125 days after inoculation.</i>
	11	3·5	2	Died of disease on 18th day : itch.
	12	3	1	Allowed to die : relapse not treated.

From the above it will be seen that five out of these 12 rats are alive and well at periods varying from 125 to 134 days. This salt is much more soluble than either the potassium or sodium compound, which may, perhaps, as well as its greater antimony content, account for its greater effectiveness. It is, however, more irritating subcutaneously in watery solution.

Experiments with Antimony upon Dogs.

In order to see what the effects of antimony would be on the larger and more important animals when suffering from trypanosomiasis, a series of experiments on dogs has been begun. The trypanosome used was that of Surra, which kills dogs of about 20 lbs. in weight in approximately 14 days, as this is the trypanosome which is of practical importance with regard to dogs.

Dr. MacConkey, of the Lister Institute, kindly performed some initial experiments for us. He made some experiments with a 20 per cent.

suspension of sodium antimony tartrate in Col. Lambkin's medium, in order to find the minimum lethal dose, and he found that 0.5 c.c. of this 20 per cent. cream per 10 lbs. of body weight was probably about the full dose. But he also found that, for practical purposes, this 20 per cent. cream was much too strong, as it caused sloughing in every case; indeed, dogs do not seem to bear these suspensions as well as they do solutions. Dr. MacConkey kept one dog alive till the 40th day, and one until the 30th, but both had many relapses.

Profiting by the experience thus gained, we have made further experiments using much smaller doses, with satisfactory results. But we have found, since trying the lithium antimonyl tartrate that this acts more effectually and with less irritation than the creams, whether of metal or salt. All the animals tabulated below are in good condition and are gaining in weight.

(Average length of untreated disease, 14 days.)

No.	Weight, in lbs.	Total quantities given, in minims.	Recur- rences.	Results to August 20.
1	22	Antimony cream, 5 per cent., 60 m. Sod. ant. tart., 2 per cent., 40 m.	2	No trypanosomes present since July 13; is alive and well on the 62nd day after inoculation.
2	22	Sod. ant. tart., 2 per cent., 80 m. Lith. ant. tart., 2 per cent., 55 m.	2	Had 9 pups during treatment; there were 42 days between the two recurrences; is alive and well on the 62nd day after inoculation.
3	37½	Sod. ant. tart. cream, 5 per cent., 80 m. Sod. ant. tart., 2 per cent., 40 m. Lith. ant. tart., 2 per cent., 90 m.	6	Incorrect dosage seems a probable cause of these relapses; is alive and well on the 62nd day after inoculation.
4	16	Sod. ant. tart. cream, 5 per cent., 40 m. Sod. ant. tart., 2 per cent., 20 m. Lith. ant. tart., 2 per cent., 40 m.	3	Is alive and well on the 53rd day after inoculation.
5	13½	Sod. ant. tart. cream, 5 per cent., 35 m. Lith. ant. tart., 2 per cent., 47 m.	3	Is alive and well on the 53rd day after inoculation.

The second and third substances mentioned in Column 3 were given at the recurrences. That these dogs are all alive and well encourages us to hope that, as we get a better knowledge of the dosage required, the recurrences may be less frequent.

Experiments made with Rats treated with Antimony, in order to find out in what Organs the Trypanosomes are latent.

The following initial experiments were made in order to find out where the trypanosomes rest during the period in which the peripheral blood is free from them, after treatment with antimony. Further experiments are being carried on for the purpose of ascertaining in what forms they are present in the organs of treated animals.

The rats were inoculated with Nagana, which is less affected by antimony than Surra, and were all treated with four doses of sodium antimony tartrate. The rats were killed at various intervals, and the organs selected (the liver and bone-marrow) were made into an emulsion with a minimum quantity of 0.75 per cent. salt-solution, and injected into other rats in doses of 1 c.c.; the same dose of blood from the heart was also given.

In the following table the signs + and — are used respectively to denote a positive or negative result.

No.	Number of days after treatment upon which rats were killed.	Blood.	Liver.	Marrow.
1	7	—	—	—
2	10	—	—	—
3	12	—	—	—
4	14	—	—	—
5	2	—	+	+
6	4	—	+	—
7	3	—	+	+
8	12	—	—	+
9	14	—	—	+
10	16	—	—	+
11	20	—	—	+

From this table it would appear that the bone-marrow is the place where the trypanosomes can live longest, and that the liver is also a place where they can find protection. This is borne out by some experiments we have made upon trypanosomiasis in birds, in which cultivations of trypanosomes can often be made from the bone-marrow when they cannot be made either from the organs or the blood. The doses given to the above rats were rather under those which we should judge to be curative, but in four cases the results were entirely negative.
